

Comparative Study of Different Vaccination Protocols Against Newcastle Disease and Infectious Bursal Disease in Broiler and Layer Chickens

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DEDICATION

To all whom I love

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LIST OF ABBREVIATIONS

AGID	Agar gel diffution test
BD	Beak dipping
DW	Drinking ater
ELD50	Embryo lethal dose 50
ELISA	Enzyme linking immunosorbent assay
HI	Heamagglutination inhibition
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus
IBV	Infectious bronchitis
I\M	Intramuscular
I\N	Intranesal
K	Komarov
MDA	Meternal driven antibody
ND	Newcastle disease
NDV	Newcastle disease virus
OIE	Office International des Epizootics
PMV	Paramyxovirus
VNT	Virus netrolization test
VV	Visrotropic velogenic

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Abstract

In the present study, two experiments were designed to evaluate different protocols of (ND) and (IBD) vaccines in broiler and layer flocks to choose the best.

In both broiler and layer chicks a total of 400 one day old chicks 200 for each, were divided in to four groups (A, B, C and D). Group D in all protocols is the control.

In broiler and layer using different ND vaccines live and inactivated vaccines in four protocols and different doses (2 doses and 3 doses) IBD D78.

In broiler the mean [ELAISA] titre (log₂) of ND in all groups after 21 days post vaccination is (8.04, 11.9, 8, 5, and 6.45) .And for IBD is (10.79, 11.41, 11.74, and 5.7).

In layer the mean [ELAISA] titre (log₂) of ND in all groups after 21 days post vaccination is (12.65, 13.28, 13.1and 4.1). And for IBD is (11.57, 10.58, 11.48, and 5.5).

In all groups in broiler, concluded that live ND with inactivated in day old and boostring with lasota in18 days is the best .Also 2doses D78 is better than 3 doses.

In layer, concluded that live ND with inactivated in day old and boostring with lasota or Komarov in3weeks and komarov with inactivated in16weeks at point of lying is the best. Also 2 doses D78 is better than 3 doses.



A, B,)

400

D

(D C

200

)

D87 (

21 ()

.6.45 8.51 11.09 8.04

.11.7 11.74 11.41 10.79

21 ()

.6.52 13.1 13.28 12.56

.11.42 11.48 10.58 11.57

18 lasota

D87

21 komarov lasota

16 komarov

D87

.

Introduction

Newcastle and infectious bursal disease are major problems in poultry industry, and have world distribution; they are most contagious viral disease. ND is caused by *Avian Paramyxo Virus1* and IBD caused by RNA virus.

The morbidity and mortality 'rates depend on the type and virulence of the virus strain, age, environmental condition, and reach to 100% mortality in susceptible chicken. The morbidity rate of IBD usually as high as 100% and mortality range between (10-70%).

ND was first reported in Sudan in 1951 but isolation and identification of virus done in 1964, from out break in Khartoum province. IBD observed in late December 1980 and early January 1981.

Vaccination is the most effective control measure of the highly contagious viral infections in poultry including ND and IBD. vaccination programs for both diseases includes live vaccines. ND virus vaccines are lentogenic strains (La Sota, HitchnerB1, clone 30) and mesogenic strains (Komarov) for secondary vaccination of birds and inactivated vaccines. IBD virus vaccines are live (D78, 228E) and inactivated vaccines.

Several epidemics of ND and IBD appeared in vaccinated chickens and caused high mortality rates. The present study was designed to find the most

suitable vaccination program against both viral diseases through the application of different vaccination programs. Another objective of this study is to find a relationship between imported vaccines and isolated pathogenic strains.

CHAPTER I

LITERATURE REVIEW

1. 1. Newcastle Disease

1.1.1. Definition

Newcastle Disease (ND) is a major viral disease of poultry and other avian species (Alexander 1991). It is enzootic in most countries in Africa, Asia and South America (Bread and Wilkes 1985). However the disease have economic impact on poultry industry precipitated following outbreaks of the disease (Alexander and Aldous, 2001).

1.1.2. History

The disease was first described by Dolye (1926) and it was named for the city in England where it is catastrophic. The disease also occurred in Java in Indonesia in the same year (Dolye, 1927).

1.1.3. The disease in Sudan

ND was reported at the first time in Sudan in 1951(Anon, 1950-1951). The virus was first isolated in Sudan in 1964 in a natural outbreak (Karrar and Mustafa 1964). Twelve of the NDV strains were isolated from the country during 1963-1979 eight of them were velogenic and four of them were mesogenic (Ballouh et al, 1983).

According to Haroun *et al.* (1992) there were four NDV strains isolated from outbreaks around Shambat village. Moreover the local isolate of NDV was first characterized by Eisa (1979). The isolate was found to be virulent ND virus. However Khalfallah *et al.* (1992) reported that the most prevalent NDV strain in the Sudan is velogenic viscerotropic pathotype, the strain, which cause heavy annual losses of the countrys poultry industry. The lentogenic strain isolated for the first time in the Sudan from apparently healthy chickens (Khalafallah, 1994)

1.1.4. Newcastle disease virus

The disease is caused by avian *paramyxo virus* genus *Rubella virus* of the family *paramyxo viridae* (Murphy *et al.*, 1999). Furthermore the family is subdivided into two sub families *paramyxo virinae* and *pneumo virinae*, the former contains the genera *Respirovirus*, *Rubella virus* and *Morbilli virus*. The later contains the genera *pneumo virus* and *Metapneumo virus* (Murphy et al, 1999). The genus *Rubella virus* consists of NDV, *Avian paramyxovirus* (2.9), canine Para influenza virus ,porcine rubella virus Michoacan Mexio virus, Mumps virus and human Para influenza viruses 2.4a and 4b (Murphy et al ,1999)

All isolates had the characteristics of NV and VV strains similar to Herts
33 Paramyxoviridae, Rhabdoviridae, Filoviridae and Bornaviridae form the

order Mononegavirales. all these families are enveloped, covered with peplomers and all have genomes consisting of a single molecule of Negative-sense, single stranded RNA. (Murphy *et al*, 1999).

1.1.5 Clinical signs

Newcastle disease virus occurs as three pathotypes Velogenic, Mesogenic and Lentogenic, these terms have come to be applied to high virulence, moderate virulence and low virulence (Alexander, 1991). Strains of NDV have been grouped into pathotype depending on clinical signs: viscerotropic velogenic is highly pathogenic form make hemorrhagic intestinal lesions, neurotropic velogenic form makes respiratory and nervous signs with high mortality mesogenic form usually occurs with respiratory signs and sometimes neural signs with low mortality. In lentogenic form there is mild respiratory signs. In asymptomatic enteric form sub clinical enteric infection and Hitchner form respiratory infections caused by viruses of lentogenic pathotype (Bread and Hansen, 1981).

1.1.6. Transmission

ND infection takes place either by inhalation or ingestion of the virus, spreading depends on the availability of the virus in an infectious form (Alexander 1988). The important route of transmission of NDV flock is aerosol (Bread and Hansen, 1984). Moreover transmission by direct contact, air aerosol, contaminated food, water and dust particles (Murphy *et al*, 1999). Infected chicks may hatch from contaminated eggs in lentogenic strain (Murphy *et al*, 1999).

In natural infections, large and small droplets containing virus will be liberated from infected birds as result of replication of the virus in the respiratory tract or as result of dust and other particles including feces (Alexander, 1991). Some authors presented evidence that wild birds were one of the major factor in the spread of NDV (Khalafalla *et al*, 1990). Vertical transmission doesn't occur as there is death of infected embryos. Also newly hatched eggs broken eggs serve as a source of virus.

1.1.7. Pathogenicity

Pathogenicity of NDV is determined by the strain of the virus, dose, route of administration, age and environmental condition. However, the disease is more acute in younger chicken and in virulent strains causes sudden death in young chickens without clinical signs, but older birds may be more protracted

and with characteristic clinical signs (Alexander, 1991b). Breed or genetic constitution of the birds appears to have very little effect on the susceptibility of chickens to the disease (Cole and Hut, 1961). Moreover, Chickens are highly susceptible, but ducks and geese may be infected and show few or no clinical signs even with strains lethal for chickens (Higgins, 1971).

1.1.8. Incubation period

Incubation period of NDV after natural exposure has been reported 2-15 days with average 5-6 days (Alexander, 1991b).

1.1.9. Immunosuppression

Immunosuppression may occur due to infection with other viruses like IBDV (Patlison and Allan, 1974). However Immunosuppression due to chicken anemia agent infection has implicated in the failure of chicken to respond well second inactivated NDV vaccine (Box *et al*, 1989).

1.1.10. NDV diagnosis:

1.1.10.1. Clinical signs

In the visotropic velogenic (VV) pathotype in chickens, clinical signs are restlessness, increase respiration, weakness prostration and death. In birds which do not die green diarrhea appear, muscular tremors, torticollis, paralysis and wing opisthotonus. Mortality reaches 100% (Alexander, 1991a)

The NV forms severe respiratory disease followed by neurological signs, dropping in egg production, morbidity is 100% ND mortality is up to 50 % and 90% (Alexander, 1991a). The mesogenic NDV causes respiratory diseases, it may cause dropping in egg production (Alexander, 1991b). The lentogenic viruses do not cause disease in adult fowl.

1.1.10.2. Gross lesions:

Gross lesions depend on the strain and the pathotype of the infecting virus and the host (Alexander, 1991a, b). The presence of hemorrhagic lesion in the intestine has been used to distinguish VVND viruses from NVND viruses (Hanson 1980). The ovarian follicles are often flaccid and degenerated. Hemorrhage and discoloration of the other reproductive organs may occur (Alexander, 1991a).

1.1.10.3. Virus isolation

Specimens for NDV isolation are taken from spleen, feces, intestinal contents or cloacal swabs, specimen taken from carcasses should reflect the clinical signs (Alexander, 1991a). Furthermore bone marrow biopsy is a useful sample in countries where transport is slow (Omojola and Hanson, 1986). In virus isolation transport the samples in frozen or chilled state is very important (Alexander, et al 1988).

1.1.10.4. Laboratory diagnosis

1.1.10.4.1. Serological tests

Newcastle disease virus may be used as antigen in a wide variety of serological test, like Hemagglutination inhibition (HI) test or enzyme-linked immunosorbent assay (ELISA) to be used for diagnosis (Alexander, 1996). Hemagglutination inhibition (HI) by sera of chickens that have ND or have been vaccinated with ND vaccines, antibodies of the sera inhibit the agglutination of fowl red blood cells, the antibodies appear 4-8 days after infection. Although HI test is simple to perform, it is difficult to standardize among laboratories (Bread and Wilkes, 1985). Other method for detecting antibodies is Enzyme Linked ImmunoSorbent Assays (ELISA). (Snyder *et al.*, 1983; Adair *et al.*, 1989). Tabidi M.H *et al.* 2004 made a comparison between the antibody (Ab) titers to the intermediate Newcastle disease virus (NDV) vaccine (komarov strain) in broiler chick using haemagglutination inhibition (HI) test and (ELISA).for all routes of the vaccine administration ,higher Ab titers were detected using ELISA technique than HI test.

1.1.11. Prevention and control

To prevent the introduction of NDV, most countries have restriction on trade in poultry products, eggs and live poultry (Alexander, 1991a). The most

important factor in preventing the introduction of the NDV and its spread is prevention at the farm level during outbreaks.

Proper vaccination programmes and strict biosecurity measures were proved in many reports as essential tools for the control of ND and IBD. (Giambrone and Clay, 1986; Wyeth and Chettle, 1990; Whitfill *et al.*, 1995; Haddad *et al.*, 1997).

1.1.11.1. ND vaccines

ND vaccines have been used successfully since 1940 to prevent this disease (Bread and Hanson, 1984). According to Alexander (1999) ideal vaccination against ND would result in immunity against infection and replication of the virus.

Commercial vaccines are live attenuated and killed vaccines.

1.1.11.1.1. Live vaccines:

ND live vaccines are lentogenic and mesogenic vaccines (Plalya and Rwegemamu, 1991). Most live virus vaccine are grown in the allatonic cavity of embryonated fowl eggs but some, not ably mesogenic strains, have been adapted to a variety of tissue culture systems (Alexander, 2004).

1.1.11.1.1.1 The lentogenic strain vaccines

These are Lasota, F (splin), Hitchner, v4 ,I2 virulent thermo stable strain (Alexander 1991a, Tuphuc, *et al.*, 1998)

Methods of application for these vaccines are drinking water, intranasal , eye dropping and beak dipping (BD) (Alexander 1991a) spray and aerosol.

1.1.11.1.2. The mesogenic strain vaccines

'These are Mukteswar ,Komarov (K) and Roakin , these are suitable only for secondary vaccination.

1.1.11.1.2. Inactivated vaccines

Inactivated vaccines are produced from infective AF treated with betapropiolactone or formalin to kill the virus and mixed with a carrier adjuvant such as aluminum hydroxide or mineral oil (Cross, 1988; Alexander 2004). Inactivated vaccines are administered by injection either intramuscularly or subcutaneously.inactivated vaccines are easier to store than live vaccines not as adversely affected by maternal immunity as live vaccines can be used in day –old chicks (Box, *et al.*,1976).They also produce low levels of protective antibodies of long duration. However, their disadvantages are that they are expensive to produce and to apply and their quality control is often difficult and mineral oils may cause hazard to the vaccinator if accidentally injected (Stones, 1979).

1.1.12. Routes of application

Methods of application of NDV vaccines are injection, eye drop, nasal

drop, drinking water or aerosol administration technique.

Drinking water is the most common means of Newcastle disease vaccination according to direction (Spalatin and Hanson, 1974). It has disadvantage, high local temperature and long time required after vaccine reconstitution may cause drop in vaccine titre, variation in the level of immunity produced in the flock (Khair, 1992).

In aerosol application it is important to achieve the correct size of particles by controlling the conditions under which the aerosol is generated (Meulemans, 1988) . The aerosol method is more efficient than intranasal route as measured by the haemagglutination inhibition test (Khair, *et al* 1998). According to Tabidi *et al.*, (1998) higher antibody titre was obtained by aerosol than by giving the same dose via the I/N route vaccination 21 days later. Further more, 80% of the first group survived virulent and virus challenge while only 60% of the second group withstood the challenge.

Some mesogenic strains are given by wing-web intradermal inoculation (Alexander, 2004).

1.2. Infectious Bursal Disease

1.2.1. Definition:

Infectious Bursal Disease (IBD) is an acute and highly contagious viral infection in chicken. (Lukert and Saif, 1991). The disease was particularly important due to high mortalities, lowered productivity among infected chicks.(Shane et al, 1994).

1.2.2. History:

The disease was first described by Gosgrove (1962), Winderfield *et al* (1962) isolated the virus and named it infectious bursal agent (IBA) .The name infectious bursal disease is termed by Hitchers (1970).

1.2.3. The disease in Sudan:

IBD was first reported in the Sudan in poultry farms in 1981 in Elobied and it was affect chicken of 6 weeks old at that time with a mortality rate of 36% (Shuaib *et al*, 1982). In Kassala the studies of IBDV revealed that the virus strain was seroepidemiologically related to those strains in Elobied outbreak (Gaffer, *et al.*, 1988). In 1986 in Sennar an outbreak of high mortality rate in 40 days old chickens (Genawi and Shuaib 1988, Genawi and Shuiab 1993). In 1990 the disease occurred in south east of Khartoum. The data presented in this outbreak revealed that a low virulent strain of IBDV is present among broiler chicks in the Sudan (Khalafalla et al 1990-1991).

Serological detection of IBD antibodies among non vaccinated, non-previously infected flocks confirms the existence of sub clinical IBD in the Sudan (Mahasin, 1998).

1.2.4. IBDV:

Infectious bursal disease virus (IBDV) is a member of the family *Birnaviridae* (Dobos *et al*, 1979). In the family three genera *Avibirnavirus*, *Aquibirnavirus* and *Entombirnavirus* ,IBDV is a member of genus *Avibirnavirus*.

The virus is resistant to many disinfectant and environmental factor for at least 4month in poultry house (Gary *et al.*, 2000). IBDV Virions are non-enveloped, hexagonal in outline with icosahedral symmetry, 60nm in diameter. The genome consists of two molecules of linear double stranded RNA (Murphy *et al.*, 1999). The larger segment encodes three proteins namely vp2, vp3 and vp4; of which vp2 and vp3 are structural proteins while vp4 is viral protease .the smaller segment encodes viral protein (vp1) 98,000 dalton and the viral polymerase (Fhey *et al.*, 1991)

1.2.5. Serotypes and Variant:

Mc ferran *et al* (1980) in European origin and Jack wood *et al* in USA (1982) presented that evidence for the presence of two serotypes designated 1 and 2 .The two serotypes are differentiated by virus neutralization test VNT ,although they show a common antigen (Saif,1994). Serotype1viruses

are only pathogenic for chicken, serotype 2 viruses infect chicken and turkey but these infections are of unknown clinical significant (Ismial, *et al.*, 1988). Immunization against serotype2 doesn't protect against sertype1 and antibodies to serotype2 are common in both chickens and turkey (Jack wood and Saif, 1983).

1.2.6. Pathogenesis of IBDV:

Infectious bursal disease is the selective replication of virus in the bursa of fabricius, which in early infections becomes enlarged up to five times its normal size and becomes edematous, hyperemic and cream colonial with prominent longitudinal lymphoid follicles of the bursa become necrotic (Murhy *et al.*, 1999). A virulence forms of the virus produce depletion of cells in the thymus, spleen and bone marrow (Alan *et al.*, 1972; Earagler *et al.*, 1974).

IBDV affects lymphoid tissue causing destruction of the bursa derived lymphocytes within the bursa of fabricius, in the thymus, spleen and caecal tonsils. Thymus derived lymphocytes are relatively unaffected (Jorden, *et al.*, 1990).

1.2.7. Transmission

The virus is resistant to heat and disinfectants, which make it, survive the adverse environmental condition: infected litter is infectious even after

seven weeks (Brar and Robinson, 1999). Equipment and people can transmit IBDV among farms (Butcher and Miles, 1999).

1.2.8. Immunity to IBVD

Birds that are recovered from IBDV infection or vaccination showed serum-neutralizing activity homologous and heterologous IBDV strains when assayed in chick embryos (Winterfield, 1969). Chicks exposed to IBVD at age of three weeks did not develop as high neutralizing titer as those exposed four weeks later (Winterfield, 1969). Carmen (1994) reported that vaccines of low virulence break down the MDA in the forth week of life. However maternal immunity is able to protect the chicks against the disease but can neutralize the vaccine virus (Vob and Vielitz, 1994; Zaheer and Saeed, 2003).

1.2.9. Immunosuppression

Dohm and Saif (1984) defined immunosuppression as a state of temporary or permanent dysfunction of the immune response resulting from damage to the immune system and leading to increased susceptibility to disease and always leading to sub-optimal antibody response (Lutticken 1997).

Immunosuppressive effect of infectious bursal disease virus on vaccination against Newcastle disease was compared two, three and four weeks old chickens inoculated with the highly virulent IBDV field isolate 90-11 and the reference serotype 1 strain GBF-1. In all ages groups, isolate 90-11

severally suppressed antibody response to Newcastle vaccination and protective vaccine immunity against Newcastle.

The mesogenic response to phytohemagglutinin of splenic lymphocytes from chicken inoculated with isolate 90-11 or strain GBF-1 was significantly lower than an inoculated control (Nokamura and Nunoya, 1992). IBD of chickens and hemorrhagic enteritis in broiler were diseases that induced immunosuppression, resulting in lowered resistance to a variety of infectious agents and poor response to commonly used vaccines (Saif, 1998).

1.2.10. Clinical signs

The signs of the disease are soiled vent feathers, ruffled feathers, watery diarrhea, anorexia, depression, dehydration and finally death (Gosgrove, 1962).

1.2.11. Lesions

1.2.11.1. Macroscopic lesions

Bursa of fabricius is the target organ for IBDV lesions (Kâufer and Weiss, 1980). It appears in the bursa before clinical signs. Gross lesion induced dehydration with darkened discoloration of pectoral muscles, intestinal mucous and renal changes (Gosgrove, 1962).

1.2.11.2. Microscopic lesions

They occur primarily in the lymphoid structures cloacal bursa, spleen,

thymus, harderian gland and caecal tonsils. Histological evidence of infection in the cloacal bursa within 24 hours (Helmloldt and Garner, 1964). Degeneration and necrosis of lymphocytes in the medullar area of the bursal follicle appears early: one day after infection. Lymphocytes are replaced by heterophilli, pyknotic debris and hyperplastic reticuloendothelial cells (Lurket and Saif, 1991). The spleen showed hyperplasia of reticuloendothelial cells in early stage then lymphoid necrosis in the germinal follicles (Lurket and Saif, 1991).

1.2.12. Diagnosis

The disease can be diagnosed by clinical signs and postmortem changes. However in practice, laboratory diagnosis of IBD depends on detection of specific antibodies to the virus, using immunological methods. Identification of the virus by direct immunofluorescent staining or direct examination by electron microscopy (McFerran *et al.*, 1980). A direct immunofluorescent smear of bursa of fabricius and direct electron microscopy examination are more sensitive method than virus isolation and direct electron microscopy (Allan, *et al.*, 1994). Agar gel diffusion test (AGID) is the most useful for detection of specific antibodies in serum or urinal antigen in bursal tissue and the test can be used to measure antibody levels (Cullen and Wyeth, 1975). AGIDT is economic to use and simple to perform and the result could

be obtained within 28 hours. Virus neutralization test (VNT) is more laborious and expensive than AGID, but is more sensitive in detecting antibodies, so it may be useful for evaluating vaccines responder or differentiate between the two serotypes (Isamil and Saif, 1990). Enzyme linked Immunosorbant assay (ELISA) was firstly developed for detection of IBDV antibodies (Marquard *et al* 1980). Antigen capture (AC) The ELISA had been used for detection of IBDV antigens directly from infected tissues (Synder *et al*, 1988). It was for serosurveyes of chicken flock (Synder *et al* 1986). The ELISA is the most rapid test and its results are easily entered into computer software. The reverse transcriptase polymerase chain reaction restriction endonuclease. (RT \ PCR – RE) assay can be used to diagnose IBD in chickens and IBDV strains (Jakwood and Nilsen, 1997).

1.2.13. Prevention and control

The control of the disease depends on vaccination and management.

1.2.13.1. Vaccination:

In 1970, only live vaccines were used to control IBD. Large variation in the degree of the attenuation of vaccines strains and the effects of heterogeneous level of maternal antibodies (which effect the suitable time to vaccinate) make many problems at that time (Myth, 1980). Maternal antibodies protect chicks for 1-3 weeks, but by boosting with oil adjuvant

vaccine, the immunity may extend to four or five weeks (Lucio and Hichner, 1979). Progeny can be protected by vaccinating the parent stock, thereby providing passive immunity, or young birds can be immunized by vaccinating with live IBD vaccine (O'Brien, 1976). Recently, technology has been developed to deliver live vaccine into eggs during the incubation period. Live vaccine virus is blended with IBD antibody and the complex is injected in ovo at 18 days of incubation. The eggs on to hatch and the vaccine virus are released when the chicks are about 7 days of age. In this way, the problem of maternally derived IBD antibody is overcome and the chicks are effectively immunized (OIE, 2000). The IBDV vaccine strains used to control the disease worldwide and classified as mild, intermediate and hot strains based on their capability to neutralize the MDA and cause.

Types of vaccines:

These are live attenuated vaccines and inactivated oil emulsion adjuvant vaccines.

1.2.13.1.1. Live vaccines

Live vaccines strains can be categorized into three groups according to the test bursal body weight ratios (Michele Guittet *et al.* 1994).

1.2.13.1.1.1. Mild strains

It is susceptible to the effect of maternally derived antibody (MDA), so should be administered only after all MDA has waned. Application is by means of intramuscular injection, spray or drinking water at 8 weeks age (Skeals *et al.* 1979).

1.2.13.1.1.2. Intermediate strains

Intermediate vaccines are used in young parent chicken if there is a high risk of natural infection with virulent IBD. They are sometimes administered at one day old as a course spray to protect chicken in the flock that may have no or only minimal level of MDA (OIE, 2000). Second and third doses are usually administered, especially when there is a high risk of exposure to virulent forms of the disease (OIE, 2000). Feed based IBDV vaccination was recently tried with good result (Hair-Bejo *et al.* 2004).

1.2.13.1.1.3. Hot strain

Hot strain is used in severe affected areas such as Intervet L_z 228E and TADLC₇₅. Its use should be where no other means of control exist (Löher, 1994).

1.2.14. Inactivated vaccine

Inactivated vaccines produce high, long, lasting and uniform levels of antibodies in breeding hens that have previously been primed by live vaccine

or by natural exposure to field virus during rearing (Cullen and Wyeth 1975).

CHAPTER II

MATERIALS AND METHODS

2.1. Chicks:

Two hundred chicks of male (Bovan Hybrid) and two hundred chicks of broiler (Ross 308) were used in the study from Coral Poultry Company.

2.2. Vaccines:

2.2.1. Clone 30:

This is Nobilis ND clone 30 is a line, freeze-dried vaccine against ND. Each dose contains at least 10^6 ELD₅₀ of the ND strain clone 30. The vaccine pellet contains stabilizers and Gentamicin.

2.2.2. ND Broiler:

Inactivated vaccine for the protection of day old chickens against ND. Nobilis MD Broiler contains one immunogenic strain of NDV. The virus has been grown on embryonated eggs and is inactivated with formalin, subsequently they have been suspended in the aqueous phase of an oil adjuvant emulsion.

2.2.3. Nobilis MA5+clone 30:

This is an alive, freeze-dried vaccine against IB and ND. Each dose contains at least 10^3 EID₅₀ IBV strain MA5 and 10^6 EID₅₀ NDV strain clone30.

2.2.4. La Sota:

It is a live NDV vaccine containing the lentogenic chick embryo propagated, Lasota strain of the virus.

2.2.5. Komarov:

It is a freeze-dried, live chick embryo adapted vaccine containing Komarov strain, supplied by the viral vaccine unit centre of Veterinary Research Laboratories.

2.2.6. Gumboro D₇₈:

Nobilis Gumboro D₇₈ is an alive, freeze-dried vaccine. Each dose contains at least 4.0 log₁₀ TCID₅₀ of the Gumboro strain D₇₈. The vaccine pellet contains stabilizer and Gentamicin.

2.2.7. Nobilis IB +ND:

This is a combined inactivated vaccine for the protection of the chickens against Avian Infectious Bronchitis (IB) and Newcastle disease (ND). It contains one immunogenic strain of IBV, one immunogenic strain of NDV, grown on embryonated eggs or Vero cell culture and inactivated with formalin subsequently they have been suspended in the aqueous phase of an oil adjuvant emulsion.

2.3. Vaccination programme:

All chicks were divided into four groups namely A, B, C and D (50 per

group). Chicks in all groups were bled by head puncture method after one day old and the collected blood left overnight at room temperature to clot and then centrifuged at 1000 rpm for 10 minutes. Separated sera were store at -20°C before tested for antibodies level for ND and IBD in 1day old using ELISA.

2.3.1. Experiment 1:

Chicks in group **A** were vaccinated at a day old with clone 30 via the aerosol and ND Broiler injection. Then, in thirteen days old, they were vaccinated with D₇₈, the second dose in 20 days and the third dose in 27 days in drinking water. Chicks in group **B** were vaccinated at a day old with MA5+clone 30+ND Broiler injection, in 13 days old vaccinated with D₇₈ and the second dose in 20 days in drinking water. Then in 23 day, they were given Lasota in DW. Chicks in group **C** were vaccinated at day old with clone 30 via the aerosol and 13 day and 20 day with D₇₈ in DW. Then, with Komarov in 23 day. Chicks in Group **D** was left without vaccination as control.

Chicks in all groups were bled by wing puncture method after 21 days and the collected blood left overnight at room temperature to clot and then centrifuged at 1000 rpm for 10 minutes. Separated sera were store at -20°C before tested for antibodies level for ND and IBD in 48 days using ELISA.

2.3.2. Experiment 2:

Chicks in group **A** were vaccinated at day old with clone 3 via the aerosol and ND Broiler injection. Then, in 24 day they were vaccinated with D₇₈, second dose in 31 days, third dose in 38 days in drinking water and finally with Komarov in 58 days. Chicks in group **B** were vaccinated at day old with MA5+clone 30 (spray) +ND Broiler (injection). Then, they were vaccinated with Lasota (DW) in 21 days. In 24 days, 31 days, and 38 days they were vaccinated with D₇₈ (DW) and with Lasota again in 58 days. Chicks in group **C** were vaccinated in day old with clone 30 (spray). In 21 days, they were vaccinated with Komarov, in 24 days with D₇₈ (DW) and in 58 days with Komarov. Chicks in groups **A**, **B** and **C** were vaccinated in 16 weeks with inactivated IB+ ND (injection). Then, a day after they were vaccinated with Komarov (intranasal) and after another week, they were vaccinated with live IB (DW). Chicks in group **D** were left as control. Chicks in all groups were bled and separated sera were used for measuring antibodies level for ND and IB 48 days using ELISA and in 19 weeks for antibodies level for ND and IB using ELISA.

2.4. ELISA for IBD and ND disease:

2.4.1 Reagents provided:

2.4.1.1 NDV and IBD coated plates:

Inactivated viral antigen on microtitre wells,

2.4.1.2, Conjugate reagent:

Sheep anti-chicken: alkaline phosphates in tris buffer with protein stabilizers, inert red dye and sodium azide preservative (0.1 % w/v).

2.4.1.3. Substrate buffer:

Diethanolamine buffer with enzyme cofactors.

2.4.1.4. Substrate Tablets

pNPP(p-Nitrophenyle phosphatase) tablets to be dissolved with Substrate buffer.

2.4.1.5. Stop solution:

Sodium Hydroxide in Diethanolamine buffer.

2.4.1.6. Sample diluent:

Phosphate buffer with protein stabilizers, inert green dye and sodium azide preservative (0.1 % w/v).

2.4.1.7. Wash buffer:

Powdered phosphate buffered saline with Tween.

2.4.1.8. Negative control:

Specific pathogen free serum in phosphate buffer with protein stabilisers and sodium azide preservative (0.1 % w/v).

2.4.1.9, Positive control:

Antibodies specific to NDV and IBDV in Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v).

2.4.2 Materials and equipment required:

Precision Pipettes and disposable tips, eight or twelve channel pipette/repeater pipette. Plastic tubes for sample, distilled or deionized water, microtiter plate reader with 405nm filter, microtiter plate washer.

2.4.3 Reagent preparation:

2.4.3.1. Substrate reagent:

1 tablet was added to 5.5 of substrate buffer and allowed to be mixed for 3 minutes or until fully dissolved. The prepared reagent was made on the day of use, it was stable for one week when kept in the dark at + 4°C. Tablets were dropped into clean container and appropriate volume of substrate buffer .was added.

2.4.3.2. Wash buffer:

The contents of wash buffer sachet were dissolved in one litre of distilled/deionized water. It remained stable for up to one month when stored in + 4°C. All other kit components are ready to use, they were allowed to

come to room temperature before use.

2.4.3.3. Sample preparation:

Test sample was diluted 1:500 by adding 1 µl to 0.5ml of sample diluent. Well was mixed with vortexing. A fresh pipette was used for each separate sample. The dilution tube was clearly identified with sample number. Positive and negative controls do not require diluting.

2.4.3.4. Test procedure:

NDV or IBDV coated plates were removed from sealed bag and the location of sample was recorded on template. 100 µl of negative control was added into wells C1 and D1. Then diluted samples were added into appropriate wells and the plate was covered with lid and incubated at room temperature (22- 27°C) for 30 minutes. The contents of wells were aspirated and the plate was washed four times with wash buffer, inverted and tapped firmly on absorbent paper. 100 µl of conjugate reagent was added to each well, and the plate was covered with lid and incubated at room temperature for 30 minutes. The wash procedure was repeated, 100 µl of prepared substrate reagent was added to each well and the plate was covered with lid and incubated at room temperature for 15 minutes. 100 µl of stop solution was added. The microtitre plate was blanked in air and the reading was recorded by reading at 405nm.

2.5. Data analysis:

Coefficient of Variation (CV): is an indicator of individual value dispersal with regards to titer mean.

$$\text{Coefficient of Variation} = \frac{\text{standard deviation}}{\text{Arithmetic mean titer}}$$

CV values is currently made according to the following threshold

< to 30% = Very homogenous 30 to 50% = Homogenous

50 to 80% = poorly homogenous > 80% = Heterogeneous

> to 150% = Very heterogeneous

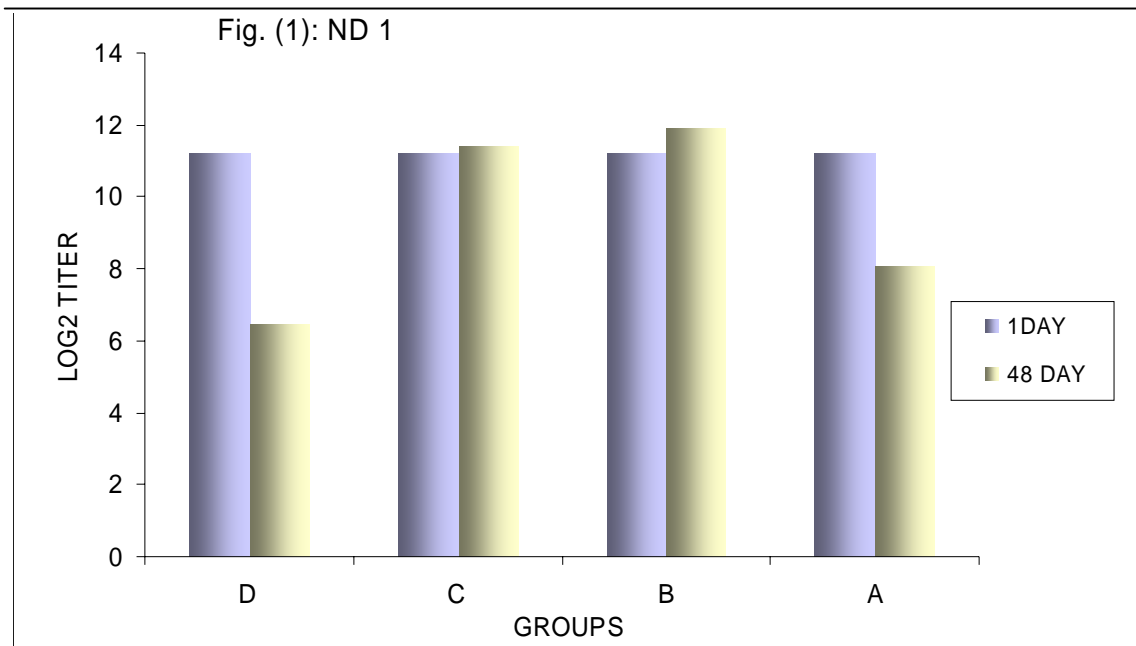
CHAPTER III

Results

3.1. Experiment 1 – Broiler flock

3.1.1. Mean antibody titre (log 2) for ND in broiler chicks

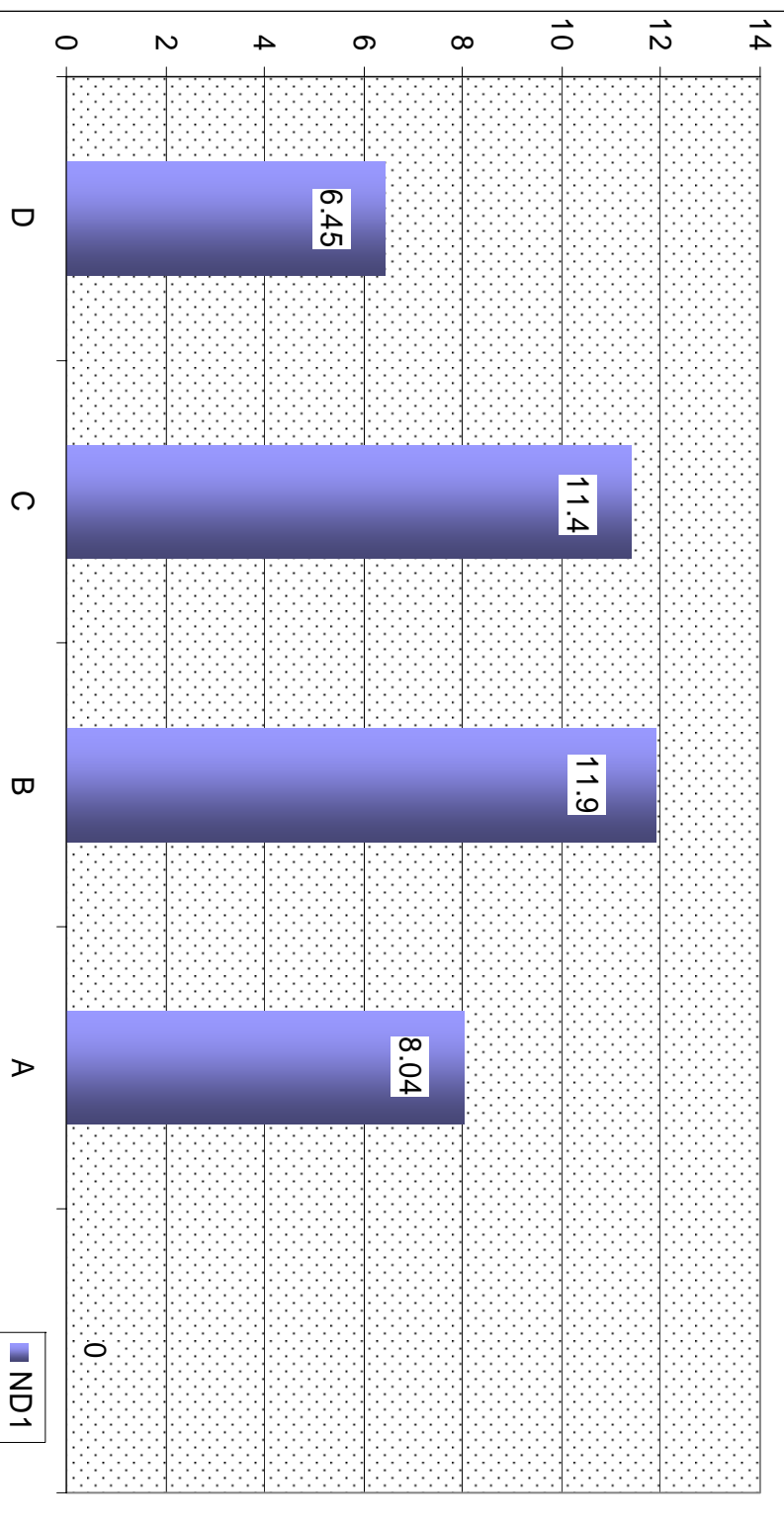
Figure 1 in experiment 1 shows mean antibody titre (log2 titre) for ND of 4 different protocol (A, B, C and D) pre and post vaccination after 21 days from the last dose by ELISA.



NB:

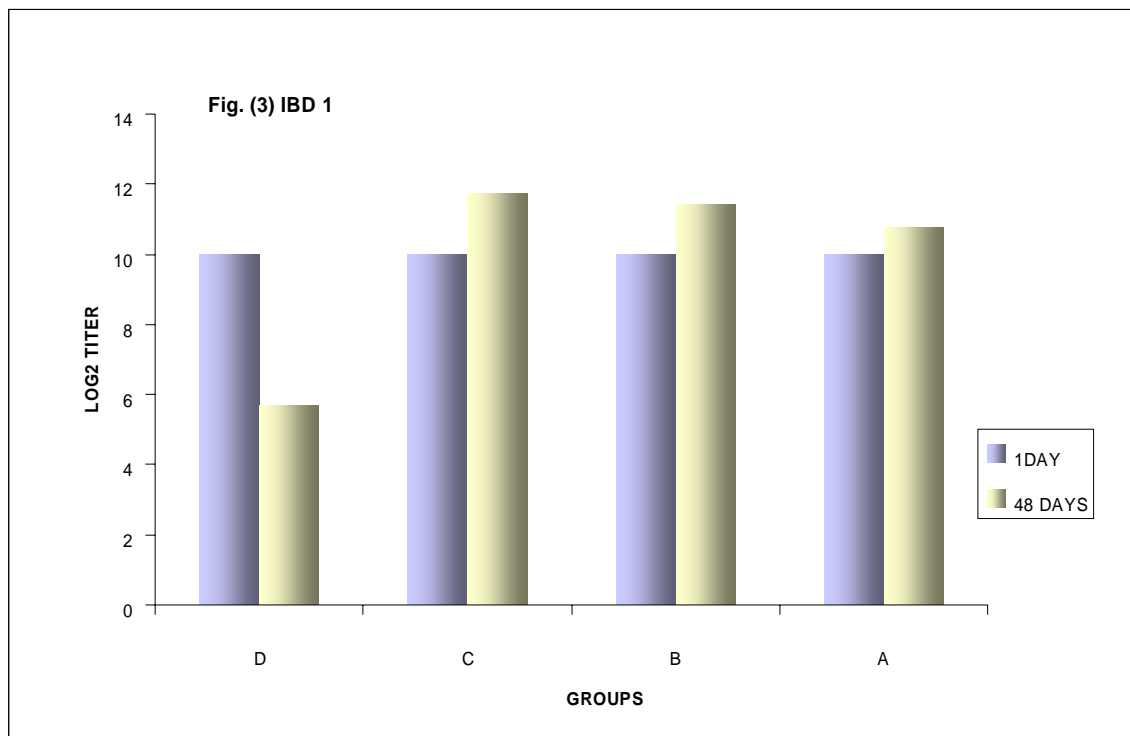
A	B	C	D
* Day 3: clone 30 (spray) + ND broiler (I/M)	* Day 3 : clone 30 + IB (spray) + ND broiler (I/M) * Day 23 : Lasota (DW)	* Day 3 – clone 30 (spray) Day 23 : Kamarov (I/N)	Non-vaccinated (as a control)

Fig.2: Log2 titre for ND1 in groups A, B, C and D in 48 days in Broiler Chicks



3.1.2. Mean antibody titre (log 2) for IBD in broiler flock

Figure 3 in experiment 1 shows mean antibody titre (log 2 titre) for IBD 1 of 4 different protocol A, B, C and D pre and post vaccination after 21 days from the last dose by ELISA.



NB:

A	B	C	D
* Day 13 – first dose D78 (DW) * Day 20 – second dose D78 (DW) * Day 27 – third dose D78 (DW)	* Day 13 – first dose D78 (DW) * Day 20 – second dose D78(DW)	* Day 13 – first dose D78 (DW) * Day 20 – second dose D78 (DW)	Non-vaccinated (as a control)

Fig 4: log2 titre for IBD1 in groups A, B, C and D In 48 days in Broiler chicks

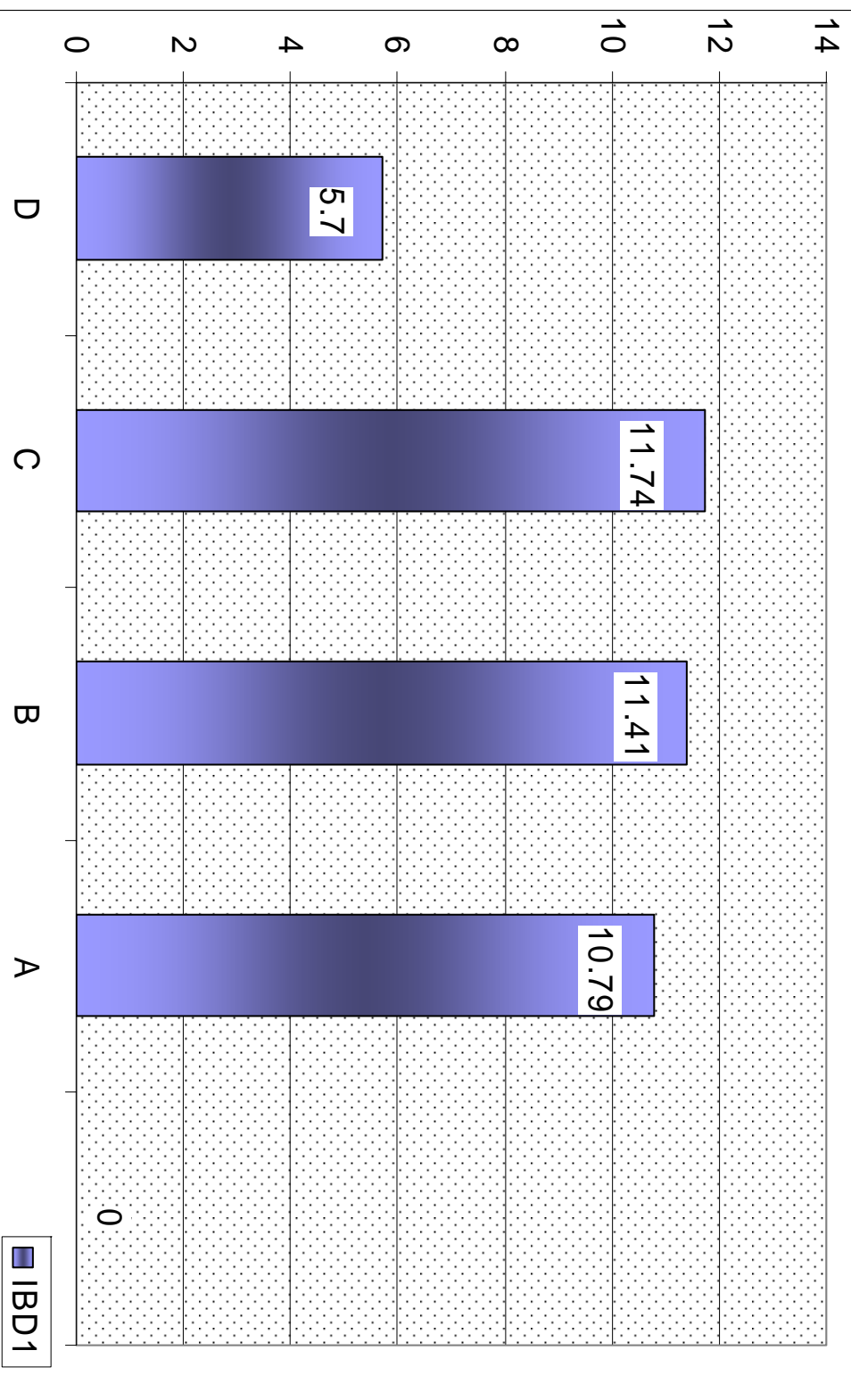


Table 1: the coefficient variation of ND MDA in Broiler Chicks

vaccinated via different protocol in groups (A, B, C and D):

Days	Group A	Group B	Group C	Group D
2 days old	52	52	52	52
48 days old	83	75	99	163

Table 2: the coefficient variation of IBD MDA in Broiler Chicks

vaccinated via different protocol in groups (A, B, C and D):

Days	Group A	Group B	Group C	Group D
2 days old	67	67	67	67
48 days old	77	42	46	50

< to 30% = Very homogenous

30 to 50% = Heterogeneous

50 to 80% = poorly homogenous

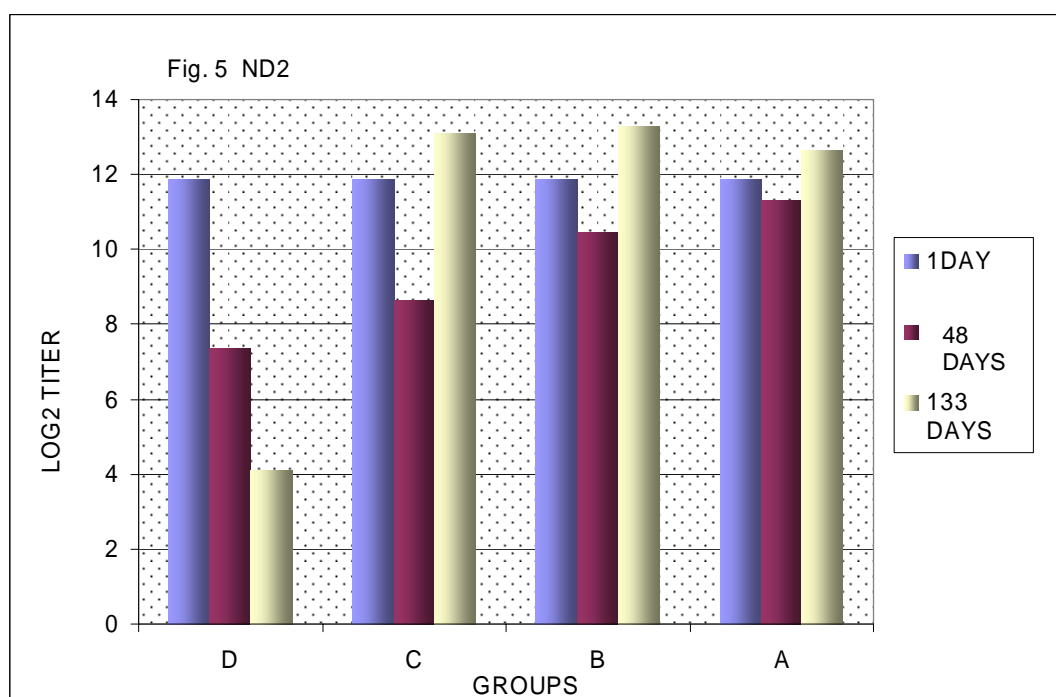
> 80% = Heterogeneous

> to 150% = Very heterogeneous

3.2. EXPERMENT 2 – Layer flock

3.2.1. Mean antibody titre (log 2) for ND in Layer flock

Figure 5 in experiment 2 shows mean antibody titre (log 2 titre) for ND2 of 4 different protocol (A, B, C and D) pre and post vaccination after 21 days from the last dose by ELISA.



A	B	C	D
* Day 3: clone 30 (spray) + ND broiler (I/M) * Day 28 Komarov (I/N) * Day 58: Komarov (I/N) * Day 133 Komarov (I/N) + (IB + ND) (I/M)	* Day 3 : clone 30 + IB (spray) + ND broiler (I/M) * Day 21 : Lasota (DW) * Day 58: Lasota (DW) * Day 133 Komarov (I/N) + (IB + ND) (I/M)	* Day 3 – clone 30 (spray) *Day 23 : Komarov (I/N) * Day 58: Komarov (I/N) * Day 133 Komarov (I/N) + (IB + ND) (I/M)	Non-vaccinated (as a control)

Fig 6: Log2 titre for ND2 in groups A, B, C and D in 48 days in layer Flock.

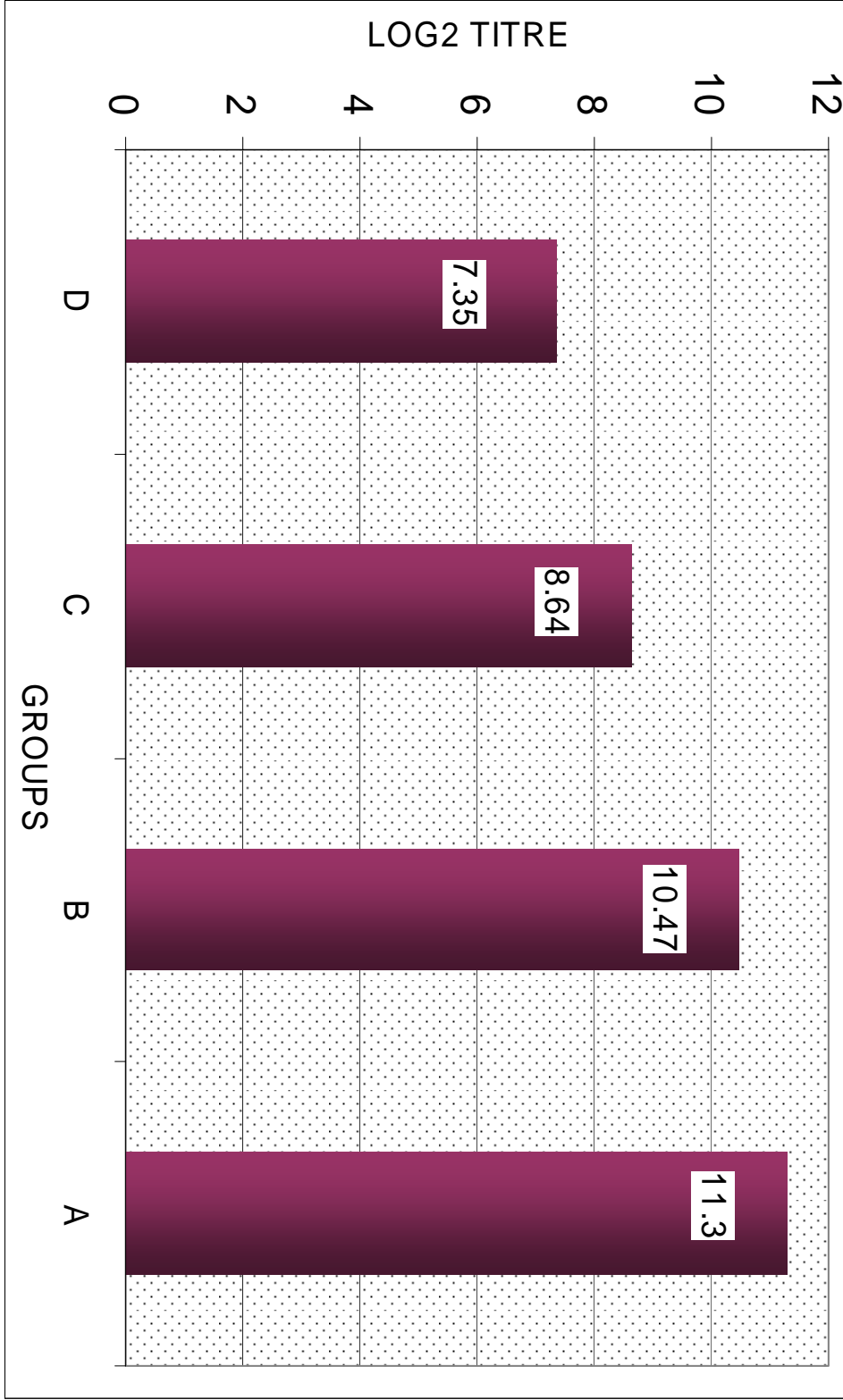
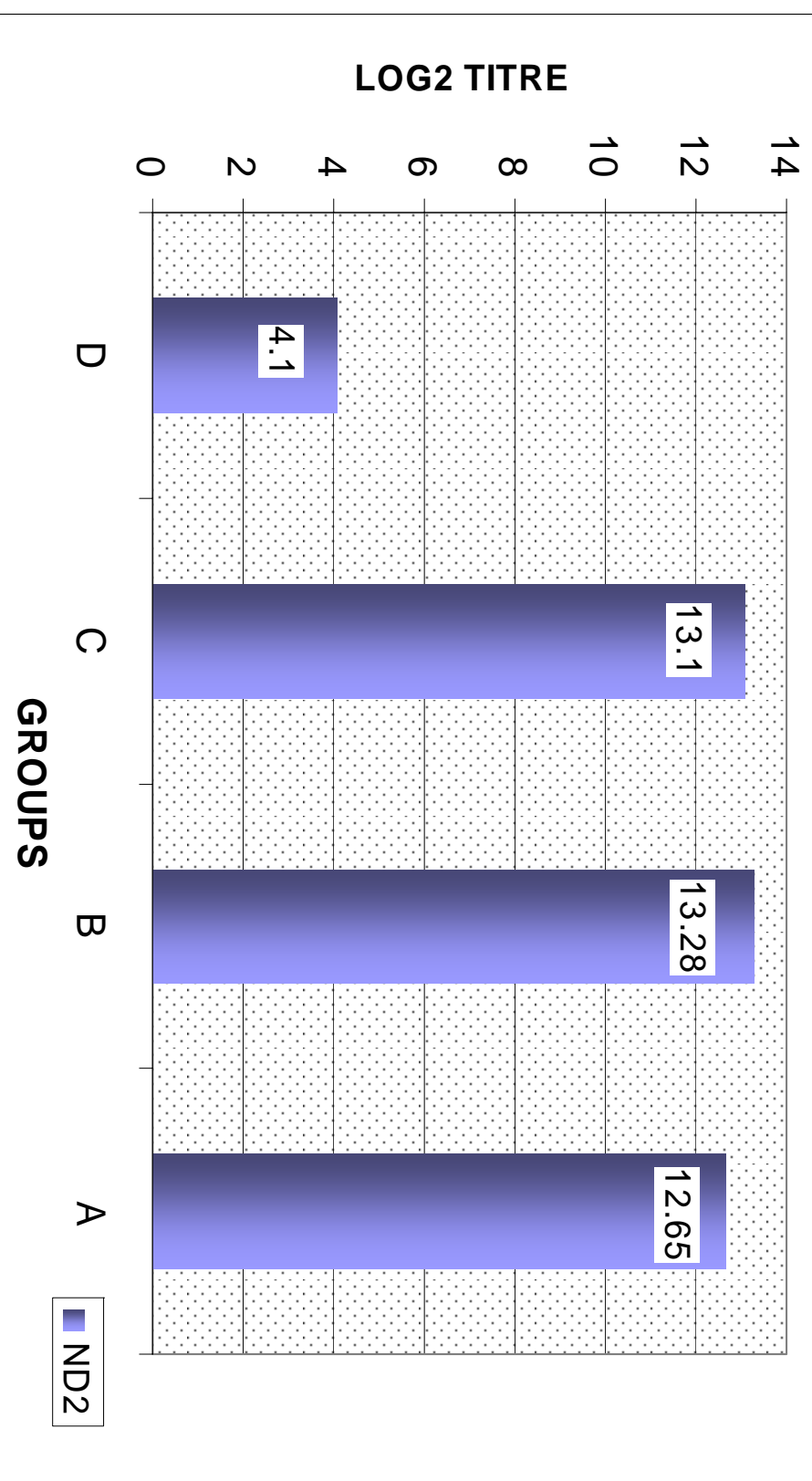
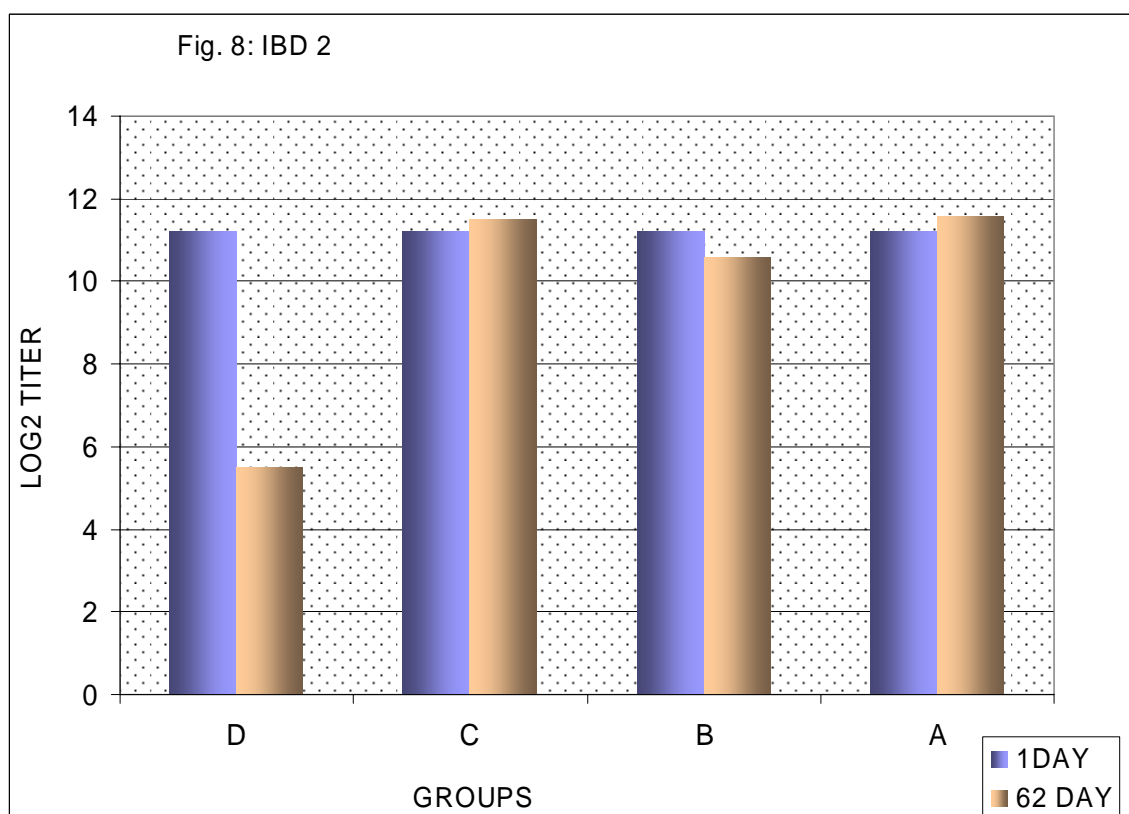


Fig 7: Log2 titre for ND2 in groups A, B,C and D in 133 Days in Layer Flock



3.2.4. Mean antibody titre (log 2) for IBD2 in Layer flock

Figure 8 in experiment 2 shows mean antibody titre (log 2 titre) for IBD2 of 4 different protocol (A, B, C and D) pre and post vaccination after 21 days from the last dose by ELISA.



NB:

A	B	C	D
* Day 24 – first dose D78 (DW) * Day 31 – second dose D78 (DW) * Day 38 – third dose D78 (DW)	* Day 20 – first dose D78 (DW) * Day 28 – second dose D78 (DW) * Day 35 – third dose D78 (DW)	* Day 24 – first dose D78 (DW) * Day 31 – second dose D78 (DW)	Non-vaccinated (as a control)

Fig. 9: Log 2 titre for IBD2 in group A,B,C and D in 62 days in Layer Folck

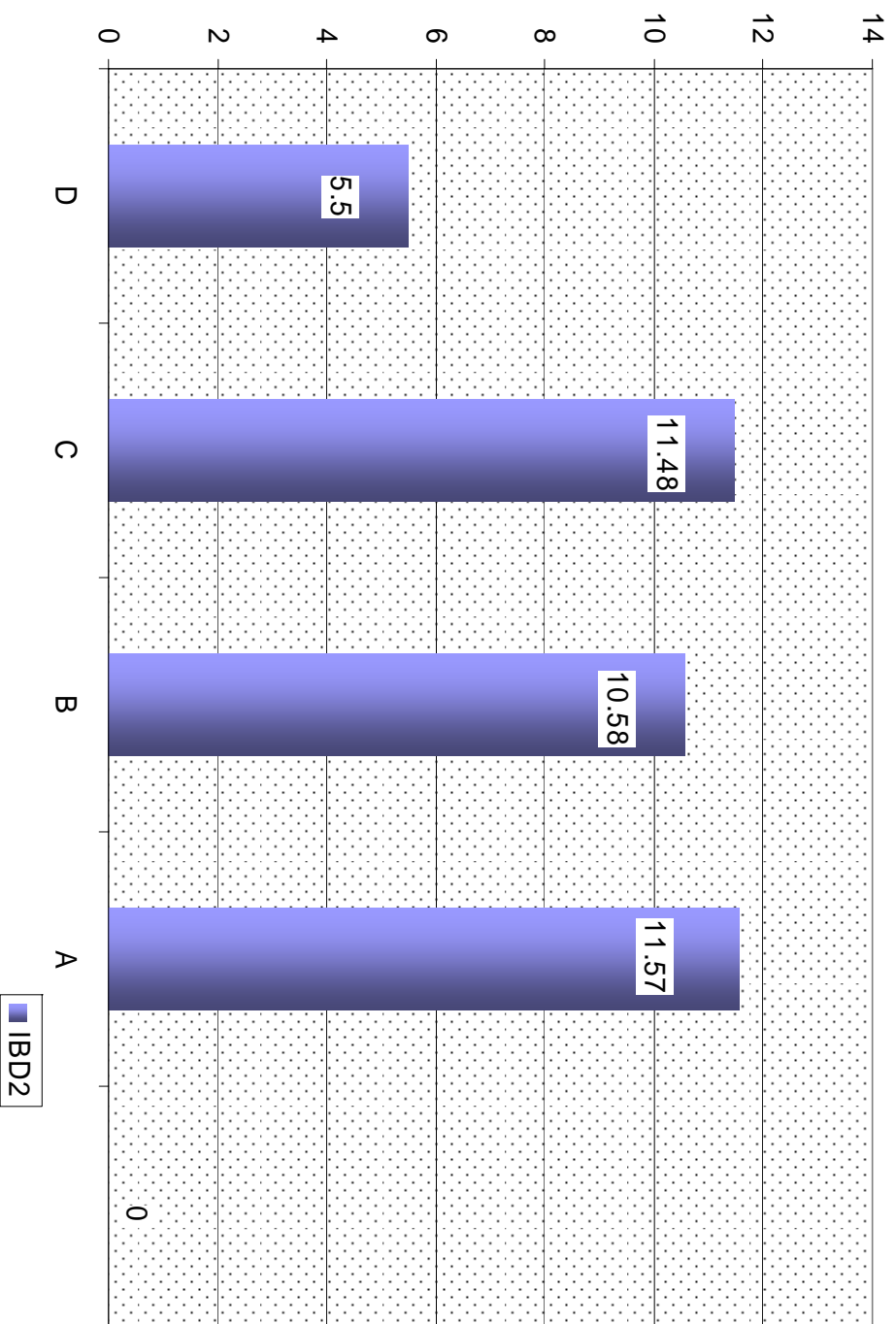


Table 3: the coefficient variation of ND MDA in Layer Chicken

vaccinated via different protocol in groups (A, B, C and D):

Days	Group A	Group B	Group C	Group D
2 days old	75	75	75	75
48 days old	82	90	200	224
133 days old	62	62	54	128

Table 4: the coefficient variation of IBD MDA in Layer Chicken

vaccinated via different protocol in groups (A, B, C and D):

Days	Group A	Group B	Group C	Group D
2 days old	62	62	62	62
62 days old	43	70	48	44

< to 30% = Very homogenous 30 to 50% = Heterogeneous

50 to 80% = poorly homogenous > 80% = Heterogeneous

> to 150% = Very heterogeneous

CHAPTER IV

Discussion

Newcastle disease and infectious bursal disease are important diseases in poultry industry in Sudan, to control these diseases many vaccines had been introduced.

The proper time of vaccination, varies and depends on maternal derived antibodies (Ab), route of vaccination, virulence of the vaccine strain, environmental stresses and management are essential factors to be considered when developing a vaccination program.

In this study we followed very important steps to choose a perfect protocol, firstly measuring MDA for ND and IBD to choose the suitable time to vaccinate this is agree with Lurket and Saif (1991) who reported that monitoring of antibody level in the Flock com aci kin determine the proper time to vaccinate . Then, using ELISA technique to measuring Ab titre according to Tabbidi *et al* (2004) who mentioned that for all routes of the vaccine administration ,higher Ab titers were detected using ELISA technique than HI test.

The satisfactory levels of immunity are not reached after vaccination of maternally immune chicks unless vaccination is delayed until the 2-3 weeks of life (Allan, 1971). So in this study the blood sample was taken in 3 weeks after vaccination to measure Ab titre after vaccination.

In ND vaccine mesogenic strains are used as booster dose because the mild live vaccines do not multiply very efficiently when used as boosters in the presence of existing immunity and hence the secondary response that they induce is rarely very much higher than the primary response. Vaccination of young chicken which have high level of MDA was considered as one of the main problems associated with control of ND (Dawson and Allan, 1973).

The effect of MDA on the immune response against ND vaccination was well documented (Mahasin *et al*, 1980; Haroun and Hajer, 1989).

In the present study, using different vaccines for ND, using live vaccines only and live with inactivated vaccines to compare. For IBD we used intermediate strain D₇₈ two doses and three doses to compare. In all protocols in this study ND vaccine was administered before IBD vaccine.

Solano and *et al*, 1989 confirmed that high level of MDA protect the chicks from infection by IBDV in early ages. In this study we used D₇₈ in 2 weeks in broiler chicks and after 3 weeks in layer chicken.

MDA was found to reduce the level of protection produced by vaccination. MDA may not always be protective against natural infection in moderate to high titer (Allan, 1973).

In experiment 1 the broiler chicks were vaccinated with ND clone 30 (spray) at one old with inactivated (ND Broiler) vaccine IM and boosting dose with La Sota vaccine (DW) at 20 days showed high Ab titer than using live only with boosting dose with Komarov vaccine. And vaccinated two doses IBD D₇₈ vaccine showed high antibody level than 3 doses.

In IBD vaccination (Turket and Saif 1991) (MDA) maternal derived antibodies were found to be sufficient to protect broiler chicks against highly pathogenic strain of IBDV during the growth period. Even the parent flock had been boosted at point of lay using oil emulsion vaccine (OIE) (Van Den Berg *et al*, 1991).

Regeswar and Masillamony (1993) mentioned that vaccinated with La Sota or B1 by aerosol at 7 days of age could be given with dose level lower than the usual dose and at early age without side effect and good immunity. The efficacy of Komarov vaccine might improve if the field dose increased (Ali, 1973). K vaccine was recommended to be given by IN in 4 weeks and repeated at point of laying. (Ali, 1978).

In Experiment (2) layer Flock using Live (clone 30) spray in day old with inactivated (NDBroiler) I\M and La Sota (DW) or Komarov 1/N at 3 weeks and in 58 day showed the highest antilgy level. In 16week using Komarov 1/N with in activated (IB + ND) I\M showed high antibody titre in all groups , also using 2 doses D78 showed high antilgy level than 3 doses

According to Khair 1992,vaccinated chicken at 4 weeks of age by Komarov strain 1/N route gave immune response greater than given vaccine by DW route. Vaccinated 18day old broiler chickens onse with Komarov1/N or1/M route had high antibody titres than DW route (Gaffer Elamin *et al*, 1993).

In conclusion, this study revealed that in broiler chicks vaccinated with clone 30 (spray) at a day old with inactivated (ND Broiler) I\M and boostring with La Sota (DW) at 20 days showed the highest Ab titer. And vaccinated with two doses IBD D₇₈ showed high antilgy level. In layer Flock using Live (clone 30) spray in day old with inactivated (NDBroiler) I\M and La Sota (DW) or Komarov 1/N at 3 weeks and in 58 day showed the highest antilgy level. In 16week using Komarov 1/N with in activated (IB + ND) I\M showed high antibody titre in all groups, also using 2 doses D78 showed high antilgy level.

Therefore it is recommended for ND to use live vaccines with the inactivated vaccines, special in layers, for IBD using 2 doses D78 after monitoring of antibody level.

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